

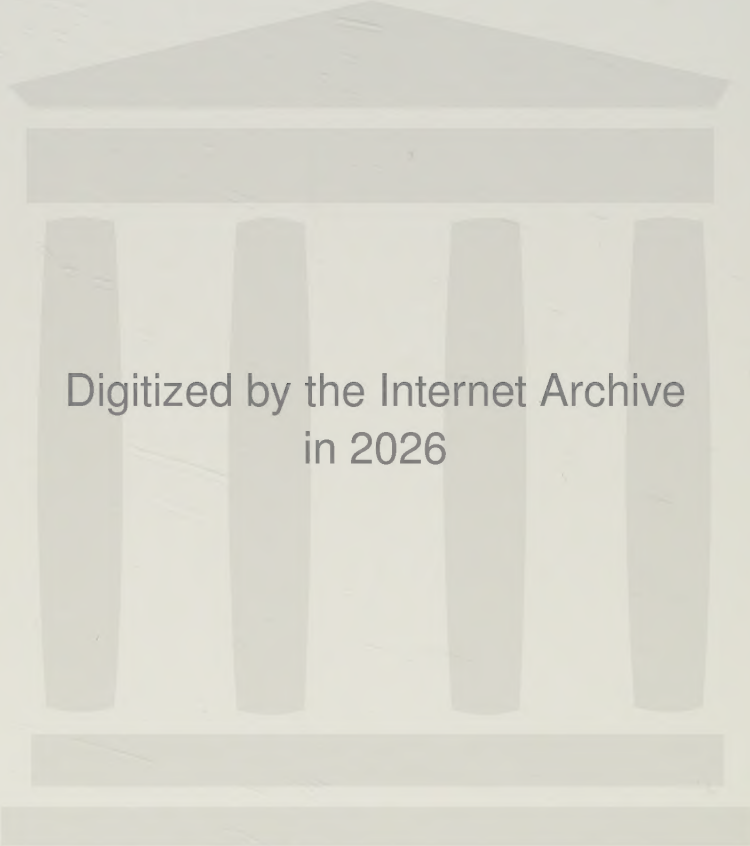
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Reaction of human dental pulp to
experimental pulpotomy and capping
with calcium hydroxide

BY ULLA SCHRÖDER

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Av
tandläkare ULLA SCHRÖDER

Department of Pedodontics
University of Lund
School of Dentistry
Malmö, Sweden



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Reaction of human dental pulp to
experimental pulpotomy and capping
with calcium hydroxide

BY ULLA SCHRÖDER

Translated by L. James Brown



The present thesis is based on the following papers:

- I* Schröder, U. and Granath, L.-E., 1971, Early reaction of intact human teeth to calcium hydroxide following experimental pulpotomy and its significance to the development of hard tissue barrier. *Odont. Revy*, 22, 379.
- II* Schröder, U., 1972, Evaluation of healing following experimental pulpotomy of intact human teeth and capping with calcium hydroxide. *Odont. Revy*, 23, 329.
- III* Schröder, U. and Granath, L.-E., 1972, Scanning electron microscopy of hard tissue barrier following experimental pulpotomy of intact human teeth and capping with calcium hydroxide. *Odont. Revy*, 23, 211.
- IV* Schröder, U. and Sundström, B., 1973, Transmission electron microscopy of tissue changes following experimental pulpotomy of intact human teeth and capping with calcium hydroxide. *Odont. Revy*, 24, preprint.
- V* Schröder, U., 1973, Effect of an extra-pulpal blood clot on healing following experimental pulpotomy and capping with calcium hydroxide. *Odont. Revy*, 24, preprint.

The papers are referred to by their Roman numerals.

INTRODUCTION

After pulpotomy it is the rule to cover the pulp wound with some sort of dressing. Calcium hydroxide paste is the dressing most widely used because it is believed to promote healing by induction of bordering hard tissue, a bridge.

Glass and Zander (1949) found a superficial area of necrosis well defined against the vital tissue and situated at some distance from the wound surface, beneath which a "dentin barrier" developed. Many other authors (Dausch and Sauerwein 1952, Nyborg 1955, Eda 1961, Svejda 1964 and Masterton 1966 and others) have also discussed the initial tissue reactions to application of calcium hydroxide, but without saying anything definite about the role played by the reactions in the development of the bridge.

Opinions differ on the significance of the bridge. Some investigators postulate that a roentgenologically or histologically demonstrable bridge implies that the pulp has healed, while others believe that a bridge may occur whether the pulp has healed or not and consequently that the bridge is of no significance for healing. The effect of calcium hydroxide on pulp and pulp healing is thus still debatable.

According to the literature, the bridge generally consists of one or more layers of different types of tissue. The pulpal layer is often called dentine (for instance by Berman and Massler 1958, Masterton 1966, Phaneuf *et al.* 1968 and Langer *et al.* 1970), dentine-like substance (Nyborg 1955) and reparative dentine (Kozlov and Massler 1960). The superficial layer of the bridge is thought to be calcified necrotic or degenerated tissue (among others Nyborg 1955, Berman and Massler 1958 and Svejda 1964). For review see *I*.

Internal dentine resorption is a frequent pathologic change in calcium hydroxide-treated primary teeth (Via 1955 and Magnusson 1970). Schröder and Granath (1971) believe that this complication may be produced by an extra-pulpal blood clot between the wound surface and the calcium hydroxide.

Despite the many investigations in animals and in man unanimity has not been achieved regarding the mode of action of the calcium hydroxide and its significance in the healing of the pulp or in the formation of a hard tissue barrier. The discrepancies may be due to the fact that insufficient attention has been given to the effect of such factors as the operative trauma, the presence of an extra-pulpal blood clot on the wound surface or the state of the pulp at the time of treatment. All the tissue changes have been ascribed mainly to the effect of calcium hydroxide.

PROBLEMS

In view of the divergence of opinions given above the following questions were posed:

Which tissue changes are produced by calcium hydroxide?

Can the tissue changes observed explain the formation of a barrier?

What does the barrier consist of?

Why is the barrier mineralised?

Are the matrix-producing cells new odontoblasts?

How does a blood clot between the wound surface and calcium hydroxide effect wound healing?

Which criteria should be used for appraising healing?

PREREQUISITES

To answer the above questions, the pulpal reactions to calcium hydroxide had to be studied under the following conditions.

1. Healthy pulp. Such a demand is necessary since an inflamed pulp would mean the introduction of an uncontrollable factor in the evaluation of the effect of the medicament. The study should thus be confined to wound healing.
2. Minimal operative trauma. This would mean that the trauma could be practically ignored. Too great a trauma may cause damage to the tissue and impair healing (Nyborg 1960, Nyborg and Halling 1963 and Mejàre *et al.* 1970).
3. Treatment of the wound. Calcium hydroxide paste should be applied in close contact with the wound surface as well as with an extra-pulpal blood clot.

MATERIAL AND METHODS

The material in *I—V* consisted of 49 teeth.

In view of the few experiments illustrating the sequence of reactions leading to wound healing and the earliest tissue changes, the material was enlarged with 27 teeth. Series A was performed in the same way as in *I* but without a 3-month-case and consisted of 10 teeth. Series B concerned short time experiments with 17 teeth at intervals of 5, 10, 15, 30 and 60 minutes, respectively.

The material thus consisted of 76 intact lower bicuspid, including 23 pairs of contralaterals. The teeth belonged to 24 girls and 28 boys, aged 10 years 6 months to 15 years 4 months, and were extracted for orthodontic reasons. Two teeth which were not contralaterals, were obtained from one boy. The total material is summarised in Table 1.

The pulps were amputated in the way described by Granath and Hagman (1971). This technique causes minimal trauma. Part of the pulp was ground off with a diamond instrument and high-speed equipment during continuous irrigation with sterile saline. The site of amputation was placed roughly at the level of the cemento-enamel junction. The pulp wound was flooded with saline until the bleeding almost stopped (*I—IV*, A and B). The sterile calcium hydroxide paste was applied in close contact to the tissue under gentle pressure. In *V* the wound was gently rinsed with saturated sterile aqueous solution of calcium hydroxide and a blood clot was allowed to develop on the wound surface before application of the medicament. In all cases with in interval longer than 30 minutes between amputation and extraction the dressing was covered with a disk of Teflon®, which was held in position by wax, and the cavity was sealed with Pharmatec® and phosphate cement or amalgam.

The operations were performed under aseptic conditions, and block anaesthesia with 1.8 ml Carbocain® was employed to avoid tissue changes in the operative field owing to the anaesthetic used and/or vascular contraction. Therefore, only lower bicuspid were used.

Before extraction all materials except the calcium hydroxide were removed, and the teeth were extracted with an elevator and/or forceps. With the teeth submerged in saline the tips of their roots were cut off with a diamond disk to facilitate the passage of the fixative into the pulp.

Light microscopy was used in all studies. Scanning and transmission electron microscopy was applied in *III* and *IV*, respectively. This was preceded by the following histologic treatment.

The fixation of the specimens was done with: formalin—glacial acetic acid—absolute alcohol (10:5:85) in *I*; 10 % neutral formalin in *II*, *III*, *V*, A and B; glutaraldehyde (4 % in 0.1 M cacodylate buffer) and postfixation in osmium tetroxide (1 % in 0.1 M cacodylate buffer) in *IV*.

The specimens in *III* were dehydrated in alcohol of increasing concentration, 70 %, 95 % and 99 %, and simultaneously treated with ultrasonics for cleaning. The specimens were coated with an approximately 200 Å thick layer of vaporised gold during rotation in a vacuum.

Decalcification was performed in 4 N formic acid, *I* and *III*, and in Versene®, *II*, *IV*, *V*, A and B.

The specimens were embedded in paraffin and cut into sections about 7 µm thick, *I*, *II*, *III*, *V*, A and B, and in Epon® and cut into 2—3 µm and ultrathin sections for light and electron microscopy, respectively, *IV*.

Selective staining was used; polychrome stain according to Herovici (1963) in *I* in order to enable distinction between precollagen (which should stain blue) and collagen (red), which was considered important for the study of the barrier formation; Mallory's P.T.A.H. for staining of fibrin in *I* and *V*; Mallory's P.T.A.H. and Weigert—van Gieson in *II*, *III* and *V* for distinguishing necrotic tissue (blue and yellow-brown, respectively) from new-formed collagen (red and yellow, respectively). Otherwise routine staining methods were applied; haematoxylin and eosin for paraffin-embedded specimens and toluidine blue (light microscopy) for Epon®-embedded specimens.

RESULTS

The results of the additional studies, A and B, were in agreement with those found in *I*.

The results are summarised in Table 1 and selected illustrations from the original papers are given in the appendix (pp. 23—33).

DEVELOPMENT AND MORPHOLOGY OF THE BARRIER

The initial changes consisted of a 3-layered necrosis.

After 5—10 minutes (Fig. 1) the connective tissue in contact with the dressing was markedly compressed, zone I. The underlying zone II showed oedema and incipient liquefaction necrosis and zone III incipient coagulation necrosis. After 1 hour zone III showed coagulation necrosis. After 3 hours the lower border of this zone constituted a line of demarcation against the underlying pulpal tissue.

During the interval 6 hours—7 days there was a slight to moderate infiltration of inflammatory cells (Fig. 2) in the residual pulp adjacent to the demarcation (*I* and A).

The superficial necrotic layer was found to contain spherical foci of calcification (Fig. 3—SC), which coalesced to form an almost homogeneous calcification zone against the underlying vital tissue (*IV*) (Fig. 3—CC).

Along the apical border of the coagulation necrosis, demarcation, the residual pulp formed collagen, which was observed after 4 days (Fig. 4). Roentgen examination showed that the barrier was radiopaque after about 7 days (*I*, *IV* and A).

Coronally the barrier was bone-like, *i.e.* collagen-rich tissue of irregular appearance with cellular and vascular inclusions (*I*, *II*, *III*, *IV* and A) (Figs. 5 a and 6). After about 1 month predentine-like tissue formed with lining cells (*I*, *IV* and A) (Figs. 5 a and 5 b). These cells produced a matrix, which was later mineralised. It was partly tubular. Judging

Table 1. *Clinical data and histologic findings.*

Interval (amp.-extr.)	minutes				hours			days					months					
	5	10	15	30	1	3	6	12	24	4	7	14	14	1	1	2	3	6
Number of cases/interval in																		
<i>I</i>			1			1	1	1	1	1	1	1		1			1°	
<i>II</i>																1	12	2
<i>III</i>																	2°	
<i>IV</i>												1			1		1°	
V														2	16			
A			1			1	1	1	1	1	1	1	1		1			
B	5	3	4	4	1													
Total	5	5	4	4	3	2	2	2	2	2	3	2	2	16	3	1	16	2 = 76
Histologic Findings																		
<i>Initial changes</i>																		
Zone I	5	5	4	4	3	2												
Zone II	5	5	4	4	3													
Zone III																		
intravascular	5	5	4	4	3													
coagulation																		
coagulation necrosis						2	2	2	2	2								
demarcation						2	2	2	2	2	2	3						
Zone IV—infiltration								2	2	2	2	1						
of inflammatory cells																		
Zone V—fibrillar limit							1	1	2	2	1							
<i>Extrapulpal blood clot</i>								1					2	16				
<i>Technically unsuccessful</i>																	2*	
<i>Hard tissue barrier</i>																		
1. necrotic tissue													2	16				
2. new-formed collagen										2	3	2						
3. bone-like tissue													2	16	3	1	16	2
4. dentine-like tissue														13	3	1	15	2
continuous														4		1	13	1
Evaluation of Healing																		
Complete														4		1	11	1
Good prospects of																	2	
Questionable														1			1	1
Bad														2	11			2*

* = same 2 cases

| = not evaluated in *I*, *IV* and A

° = also included in *II*

from their ultrastructure and function, the cells were odontoblasts (*IV*).

After 3 months the pulpal part of the barrier consisted of a mature collagen tissue with tubules (*I*, *II*, *III* and *IV*) (Figs. 6 and 8 a). This tissue was found to be predentine (*IV*) (Fig. 8 b). Scanning electron microscopy (*III*) revealed that the pulpal surface of the barrier had tubular openings, resembling dentinal tubules, but were irregularly distributed and of varying size.

The coronal part of the barrier was found to be covered by a tissue that resembled unorganised precipitate and partly disappeared after demineralisation. Underneath there were irregularly arranged collagen structures (*III*) (Fig. 7).

When a blood clot was left between the wound surface and the calcium hydroxide (*V*), the coronal layer of the barrier contained necrotic, calcified tissue which often was intermingled with new-formed collagen. Necrotic tissue was mostly observed underneath areas with the most extensive clots.

EVALUATION OF HEALING

In the evaluation of the results in *II* the following definitions were used.

Complete healing: a histologically continuous hard tissue barrier and a residual pulp without infiltration of inflammatory cells.

Good prospects of healing: a histologically continuous hard tissue barrier and a residual pulp with a minor and limited infiltration of inflammatory cells.

When calcium hydroxide was brought in direct contact with the wound, complete healing occurred in 13 out of 17 cases (76 %) and the prospects of healing were good in a further 2 cases (12 %) (*II*). In the remaining 2 cases the barriers were discontinuous, which was seen in association with active internal dentine resorption and moderate lymphocyte infiltration, respectively. The prospects of healing in these cases were considered questionable.

Roentgenologically, no difference was found between *II* and *V*. All cases had demonstrable barriers. In *V* subjective symptoms were noted, which in 2 cases made extraction necessary already after about 2 weeks. In a further 7 cases symptoms appeared varying from a brief sensation of discomfort to toothache.

Only 4 out of 18 cases (22 %) healed in *V*. The remaining 14 cases had discontinuous barriers (Figs. 9 and 10), a varying degree of infiltration of inflammatory cells and necrotic areas, particularly adjacent to the breaks in the barriers.

The difference between frequency of complete healing in *II* and *V* was statistically significant at the 1 % level according to the Chi-square analysis ($\chi^2 = 8.2$). Yates' correction was applied.

Thus, the presence of a blood clot between the wound surface and the calcium hydroxide seriously impaired wound healing by causing chronic inflammation in the adjacent residual pulp.

DISCUSSION

Tentative answers to the questions posed are discussed below.

Which tissue changes are produced by calcium hydroxide?

The pH of calcium hydroxide is about 12. On application to loose connective tissue it has a caustic effect with a multilayered necrosis as a result. Hypothetically, the development of the various zones may be as follows.

Zone I, compressed superficial layer, is a result of coronal pressure, *i.e.* application pressure and pressure due to oedema from zone II.

Zone II, oedema and liquefaction necrosis is a result of severe chemical injury. Escaped tissue and plasma proteins partly neutralise the hydroxyl ions.

Zone III, coagulation necrosis, is a consequence of pressure produced by the oedema in zone II combined with the effect of still unneutralised hydroxyl ions.

The multilayered necrosis may thus be regarded as the combined effect of caustic injury, application pressure and increased intrapulpal pressure by oedema, *i.e.* a mechanico-chemical effect.

Another cause of the initial changes might be the alkalinity as such. Eda (1961), who studied wound healing in dogs' pulps, found application of magnesium oxide to be followed by formation of a hard tissue bridge. Infiltration of inflammatory cells, intrapulpal haemorrhage and degeneration were found in the adjacent pulp. In a personal unpublished pilot study in which magnesium oxide mixed with sterile physiologic saline to paste consistency (pH about 11) was applied to the pulp

wound no chemical injury was observed. Thus, it is probable that it is the hydroxyl ions rather than the alkalinity that is responsible for the initial tissue changes following application of calcium hydroxide.

Can the tissue changes observed explain the formation of a barrier?

Judging from the low-grade infiltration of polymorphonuclear leukocytes the coagulation necrosis seals off the residual pulp from the zone of liquefaction necrosis of the superficial necrosis produced by the calcium hydroxide. It might therefore be concluded that the coagulation zone, zone III, is probably the mild irritant to which the underlying pulp reacts with collagen formation, the barrier, which after about 7 days is radiopaque.

What does the barrier consist of?

The barrier formation induced, solely by the effect of calcium hydroxide, as in *I—IV*, is two-layered with a bone-like and a dentine-like layer, respectively. The barrier consists of mineralised new-formed collagen, with cellular and vascular inclusions in the coronal layer.

The necrotic tissue observed in the barriers in *V* might thus be ascribed to the effect of the blood clot between the wound surface and the medicament and not to the effect of the calcium hydroxide.

Why is the barrier mineralised?

Hitherto no explanation has been offered for the mineralisation of the barrier. It has been generally assumed that calcium hydroxide induces hard tissue formation. But the findings in the transmission electron microscopy study (*IV*) appear to warrant the conclusion that it is the calcification of the coagulation zone and neighbouring degenerated cells that starts the mineralisation of the collagen-rich tissue in the coronal layer of the barrier. The deposition of calcium salts appears to spread into the collagen-rich tissue. Autoradiographic studies by Sciaky and Pisanti (1960) and Pisanti and Sciaky (1964) have also shown that it is tissue calcium and not calcium from the calcium hydroxide that is utilised in the mineralisation of the barrier.

Are the matrix-producing cells new odontoblasts?

The conclusion that the cells are new odontoblasts is based partly on their well developed endoplasmic reticulum and partly on their localisa-

tion in direct contact with the coronal layer of the barrier. Furthermore, the tissue adjacent to the cells resembled ultrastructurally predentine and contained cellular extensions. Apically to the cells the pulp tissue was normal.

How does a blood clot between the wound surface and calcium hydroxide effect wound healing?

A histologic investigation of pulpotomised primary molars with internal dentine resorption (Schröder and Granath 1971) indicated a relation between a blood clot on the wound surface and the resorption. The results in *V* showed that the clot or its components or degradation products interfered with wound healing by causing chronic inflammation in the adjacent pulp tissue. Fibrin is one of the components that has been shown to have a chemotactic effect on polymorphonuclear leukocytes. An increased accumulation of such cells would probably impair healing by potentiating the low-grade inflammatory reaction which normally occurs within 6 hours to 7 days following application of calcium hydroxide (*I* and *A*).

Which criteria should be used for appraising healing?

Clinically, the hard tissue border of the pulp wound is considered to be of great significance, because of the practical difficulty in artificially sealing off the residual pulp and keeping it free from infection. This decided the choice of criteria for wound healing in the present investigations. The presence of a histologically continuous hard tissue barrier was also found to be related biologically to the healing process. A residual pulp without infiltration of inflammatory cells was seen only in connection with a continuous hard tissue barrier.

The results regarding frequency of complete healing in *II* and *V* were compared despite the difference in observation time. This was considered justified on the basis of the following arguments. The initial tissue reactions, *i.e.* the multilayered necrosis, were well established within some hours after application of calcium hydroxide and no delayed effect was observed in *I* and *A*. The process of wound healing was well under way after 7 days as indicated by mineralised new-formed collagen and a residual pulp practically free from infiltration of inflammatory cells. In view of the state of the unhealed cases in *V* with discontinuous barriers and chronic inflammation and necrotic areas in the adjacent residual pulp it must be considered less likely that a longer observation time would lead to increased frequency of complete healing.

The difference between wound healing and restitution of inflammatorily changed tissue in the residual pulp must be recognized when discussing healing following therapeutic pulpotomy.

Concerning wound healing the formation of a hard tissue barrier must be considered important for an organ without epithelium, since the barrier helps to protect the pulp from external irritation. But the state of the residual pulp at the time of treatment is an equally important factor for successful therapeutic pulpotomy. The experimental amputation technique, somewhat modified, was used in an investigation of 29 therapeutically pulpotomised primary molars (Schröder unpubl.). The wound was treated in the way described in *I* and the residual pulp was clinically healthy at the time of treatment (for criteria for this diagnosis, see Schröder and Granath 1971). The treatment was judged as clinically and roentgenologically good in about 75 % after 1 year and the frequency of demonstrable barriers was 85 %. In the unsuccessful cases internal dentine resorption occurred, which under the prevailing conditions might be ascribed to a misjudgement of the state of the pulp at the time of treatment, since the high frequency of demonstrable barriers suggested that the operative trauma had been minimal and wound treatment adequate.

A clinically healthy residual pulp must be regarded as necessary for successful pulpotomy with calcium hydroxide as wound dressing, at least as far as primary teeth are concerned. Young permanent teeth with incomplete root development might have the ability to heal in spite of chronic inflammation of the residual pulp. This is because of their better nutrition.

CONCLUSIONS

The observations made in this study appear to warrant the following conclusions.

1. It is the concentration of the hydroxyl ions rather than the calcium hydroxide as such that is decisive for the initial tissue changes.
2. The initial tissue injury produced consists of a multilayered necrosis, with firm necrosis, coagulation necrosis, adjacent to vital tissue.
3. The coagulation necrosis constitutes
 - a) a mild irritant to which the residual pulp after a short interval of infiltration of polymorphonuclear leukocytes responds with formation of collagen, *i.e.* the formation of a barrier, and
 - b) owing to its calcification, it initiates mineralisation of this collagen-rich tissue, the barrier.
4. The barrier consists of 2 layers
 - a) the coronal layer is bone-like with irregularly arranged collagen fibrils and cellular and vascular inclusions.
 - b) the pulpal part is dentine-like and develops after about one month immediately adjacent to the coronal part of the barrier. After about 3 months the tissue formed last resembles predentine, *i.e.* it consists of densely packed collagen fibrils and cellular extensions.
5. The matrix-producing cells along the barrier are observed after about 1 month and structurally and functionally they appear to be odontoblasts. Their localisation, in direct contact with the coronal layer of the barrier, indicates that they are new odontoblasts.
6. The presence of a blood clot between wound surface and calcium hydroxide interferes severely with wound healing by causing chronic inflammation in the adjacent pulp.

7. A continuous hard tissue barrier appears to be biologically connected with complete healing, since residual pulps without any infiltration of inflammatory cells were seen only in association with such barriers. It must be considered favourable to use a dressing that induces hard tissue formation.

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APPENDIX

Fig. 1. 10 minutes, *I*; compressed superficial layer, zone I; oedema and incipient liquefaction necrosis, zone II; intravascular coagulation and incipient coagulation necrosis, zone III; polychromic, $\times 109$.

Fig. 2. 24 hours, *I*; dissolution of necrosis and moderate infiltration of polymorphonuclear leukocytes in the adjacent pulp tissue; polychromic, $\times 109$.

Fig. 4. 7 days, *I*; broad zone of new-formed collagen; polychromic, $\times 109$.

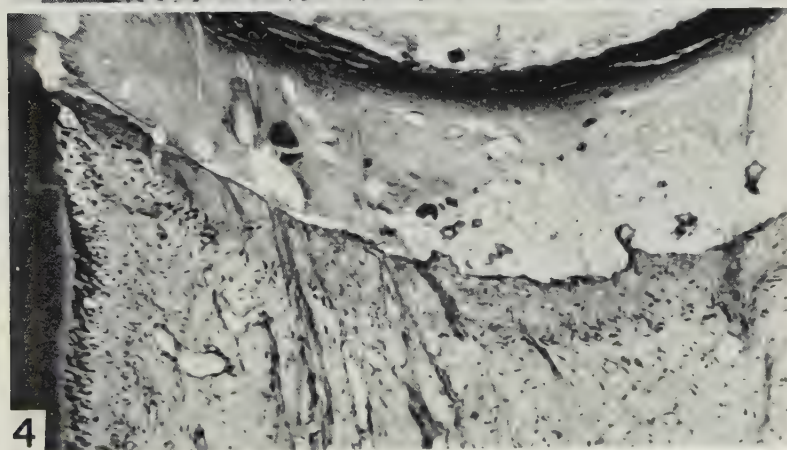
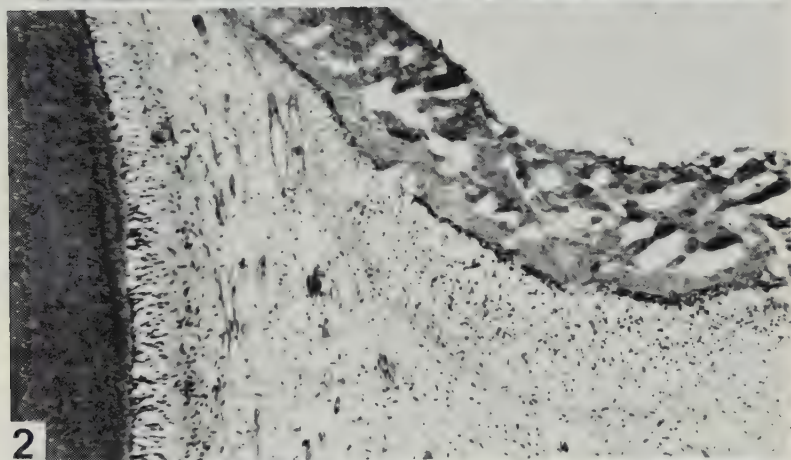


Fig. 3. 7 days, *IV*; N = necrotic zone, P = pulp tissue, SC = spherical calcification, CC = confluent calcification front, DC = degenerated cell with spherical bodies (SB); $\times 14,100$.

Fig. 6. 3 months, *II*; different layers of the barrier, bone-like tissue (I) and dentine-like (II); necrotic debris (arrow) from the lowermost zone of the superficial necrosis caused by calcium hydroxide; htx-eosin, $\times 120$.

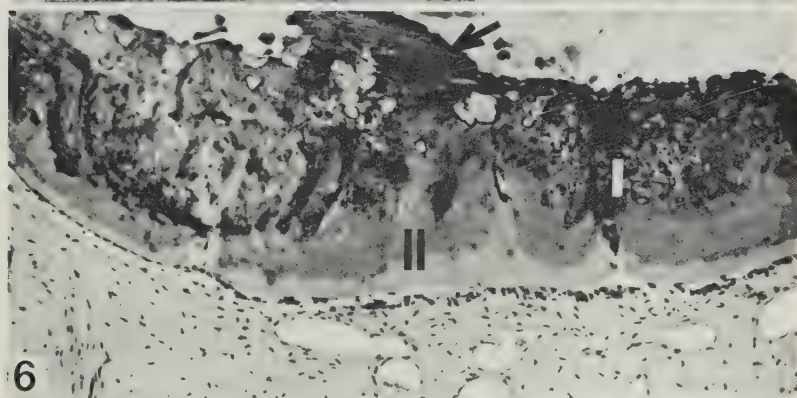
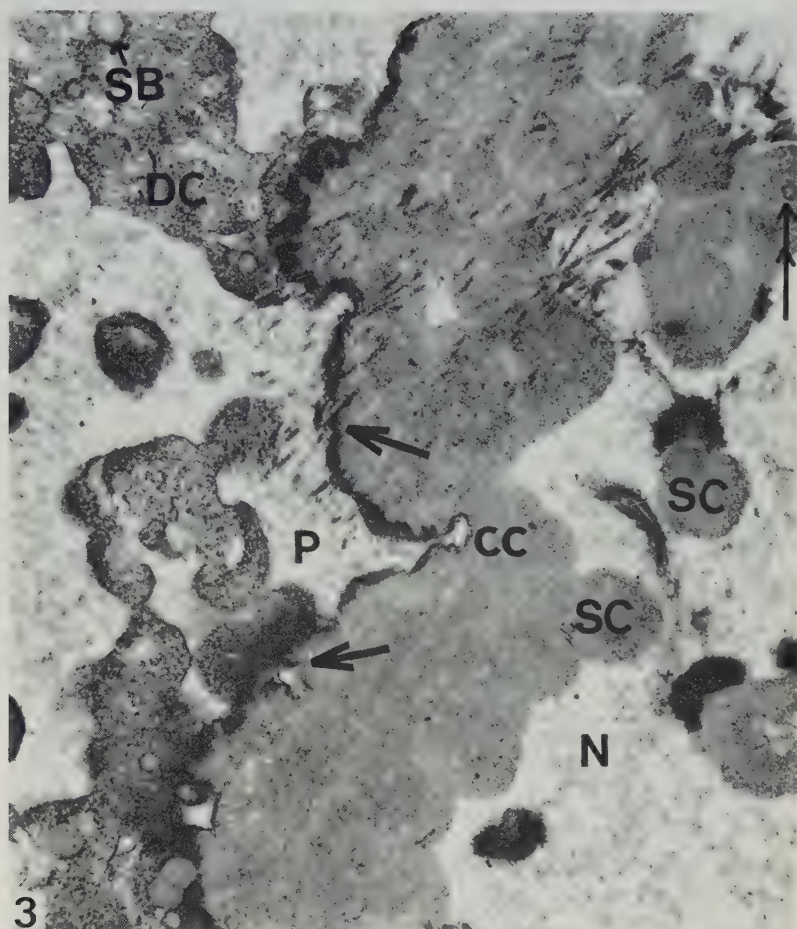


Fig. 5. 1 month, *IV*; a) showing barrier with lining cells; $\times 520$, b) lining cells showing a well developed rough-surfaced endoplasmic reticulum (RER), newly deposited collagen fibrils (NC), predentine-like tissue (PD) with densely packed collagen fibrils and cellular extension (CE); $\times 12,500$.

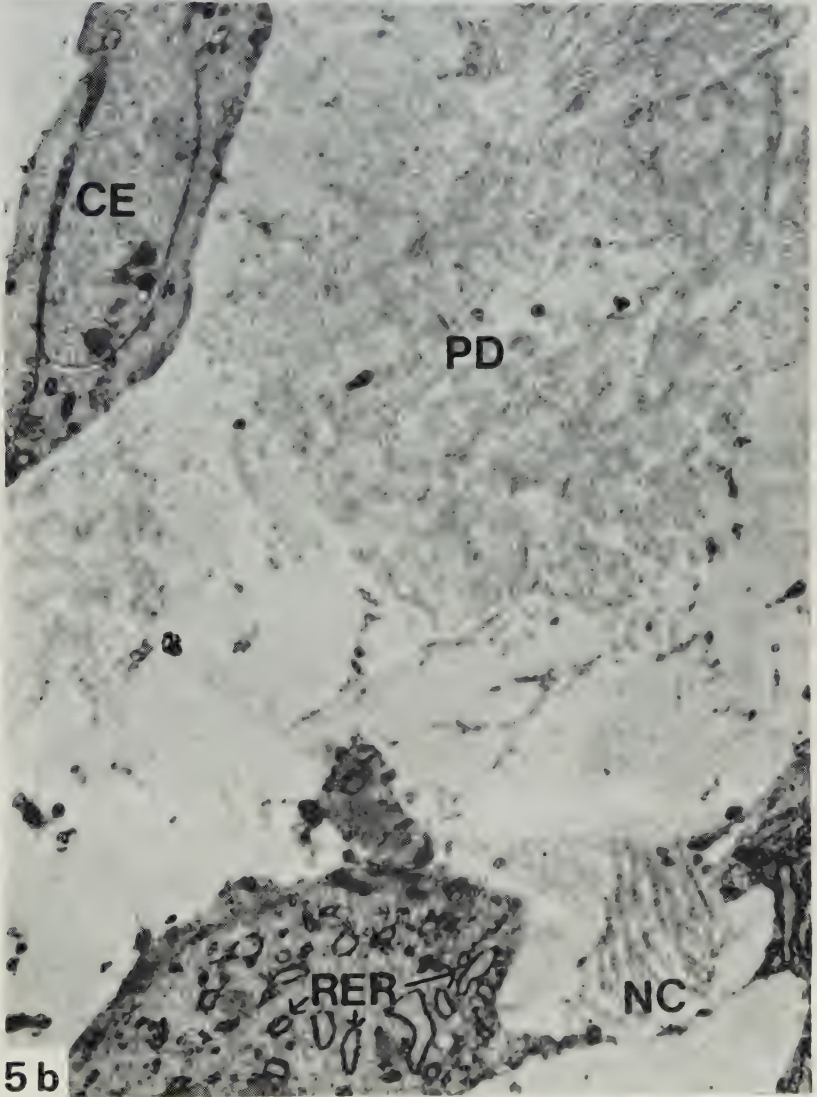
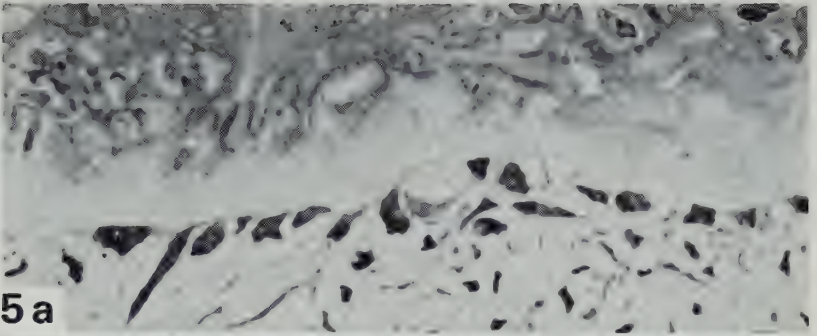


Fig. 8. 3 months, *IV*; a) barrier with elongated lining cells; $\times 660$, b) predentine (PD) with cellular extensions (CE); $\times 17,800$.

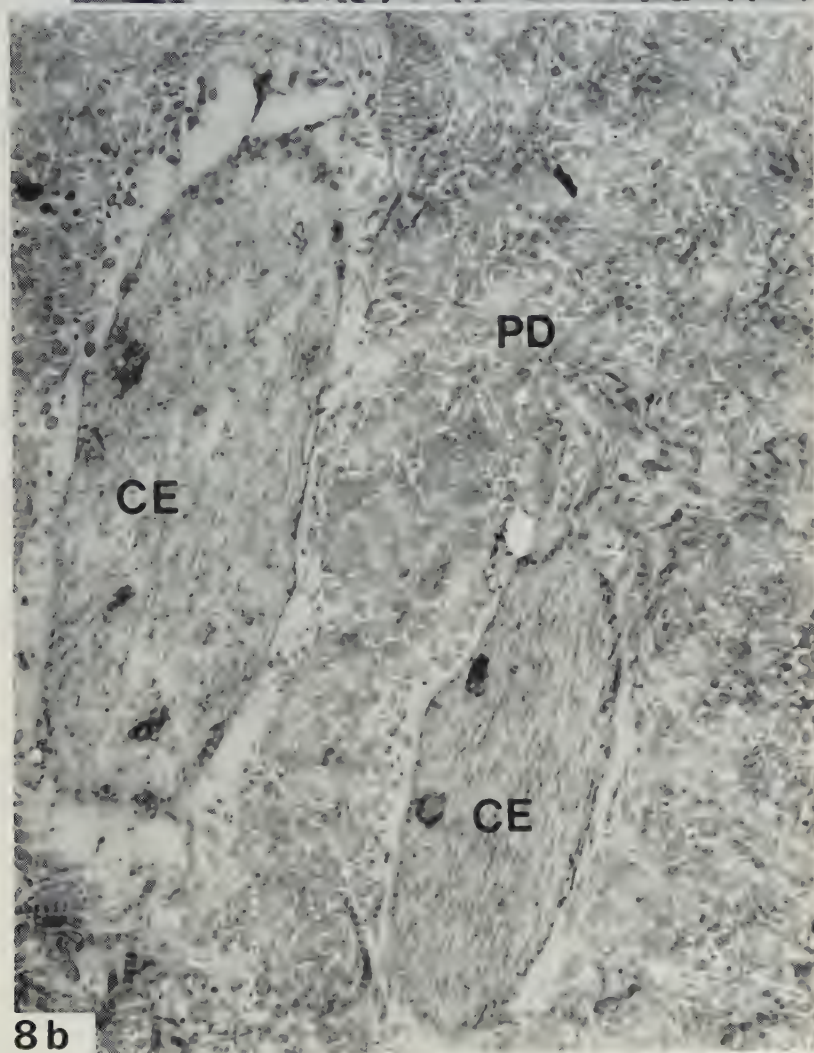
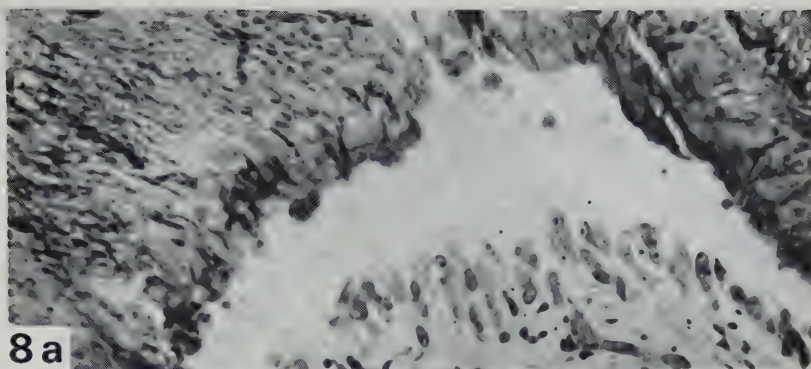


Fig. 7. 3 months, *III*; coronal surface of the barrier after demineralisation; collagen structures are seen (arrow) while superficial tissue appears structureless (double arrows); $\times 5,600$.

Fig. 9. 1 month, *V*; discontinuous barrier; necrotic area in the adjacent pulp (n) and a moderate cellular infiltration; remnants of the superficial necrosis (I) and blood clot (II); Weigert—van Gieson, $\times 50$.

Fig. 10. 1 month, *V*; discontinuous barrier; dense cellular infiltration in the adjacent pulp; remnants of the superficial necrosis (I) and the blood clot (II); Weigert—van Gieson, $\times 35$.

